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Biosynthesis of Monobactam Compounds: Origin of the Carbon Atoms in the β-Lactam Ring

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The biosynthesis of monobactams by strains of *Chromobacterium violaceum*, *Acetobacter* sp., and *Agrobacterium radiobacter* was studied. Monobactams were produced during logarithmic growth by *C. violaceum* and *Acetobacter* sp. and during late log growth on glycerol and in the stationary phase by *A. radiobacter*. The addition of various amino acids failed to significantly stimulate monobactam production in any of the producing organisms. Several ^{14}C-amino acids and pyruvate were incorporated in vivo into monobactams. Serine, glycine, and cysteine were better incorporated than alanine or aspartate, whereas an excess of nonradioactive serine depressed the incorporation of labeled cysteine, glycine, and pyruvate. A comparison of [1-^{14}C]glycine and [2-^{14}C]glycine incorporation data suggests that glycine was first converted to serine. With a mixture of [*U*-^{14}C]serine and [3-^{3}H]serine, *C. violaceum* synthesized a monobactam with a complete retention of tritium, whereas with a [*U*-^{14}C]cystine and [3-^{3}H]cystine mixture, there was an extensive loss of C-3 tritium. *Acetobacter* sp. and *A. radiobacter* also utilized the double-labeled serine without the loss of tritium in their respective monobactams. It appears, therefore, that in the three organisms, the carbon atoms of the β-lactam ring of the monobactam are derived directly from serine without the loss of the C-3 hydrogen atoms, probably by an S_N2 ring closure mechanism. With [*methyl*-^{14}C]methionine, most of the radioactivity in the monobactam from *Acetobacter* sp. was in the methyl moiety of the β-lactam ring methoxyl group.

A group of novel monocyclic β-lactams of bacterial origin called monobactams was recently described (17). Monobactams are *N*-acyl derivatives of 3-aminomonobactamic acid (Fig. 1). Most naturally occurring monobactams possess a 3α-methoxy group, but a desmethoxy monobactam is known (17). SQ 26,180 (Fig. 1) is produced by certain strains of *Chromobacterium violaceum*, and SQ 26,445, which has a γ-linked D-glutamyl-D-alanyl side chain (Fig. 1), is produced by numerous strains of *Gluconobacter* and *Acetobacter* (17). SQ 26,445 was independently isolated from a strain of *Pseudomonas acidophila* and given the name sulfazecin (6). Certain strains of *Agrobacterium radiobacter* produce a mixture of monobactams, from which five closely related substances that differ in the number of sulfate groups on the 3-acyl substituent and in the presence or absence of a methoxyl group have been isolated (17). The predominant compound in defined medium is SQ 26,812 (Fig. 1).

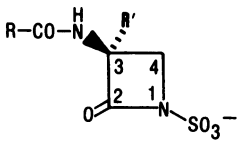
This study describes the production of monobactams and the characteristics of radiolabeled precursor incorporation in three different bacteria. The carbon atoms of the β-lactam ring of the monobactams are shown to be derived from serine, and incorporation takes place with retention of the C-3 hydrogen atoms of serine, probably by a direct S_N2 ring closure mechanism. The methyl portion of the methoxyl group of SQ 26,445 is derived from methionine.

(A preliminary account of this work has been presented [J. O'Sullivan, C. A. Aklonis, A. M. Gillum, M. L. Souser, and R. B. Sykes, Program Abst. Intersci. Conf. Antimicrob. Agents Chemother. 21st, Chicago, Ill., abstr. no. 877, 1981].)

MATERIALS AND METHODS

Radioactive materials were obtained from Amersham Corp., Arlington Heights, Ill., ICN, Irvine, Calif., or New England Nuclear Corp., Boston, Mass. Other chemicals were analytical grade, when possible, and were usually obtained from Sigma Chemical Co., St. Louis, Mo. X-ray film was obtained from Kodak, Rochester, N.Y. Econofluor and Aquasol scintillation fluids were obtained from New England Nuclear.

The monobactam-producing strains used in this study were *C. violaceum* ATCC 31,532, SC 11,378; *Acetobacter* sp. ATCC 21,780, SC 11,662; and *A. radiobacter* ATCC 31,700, SC 11,742 (SC, Squibb Collection).



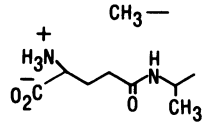
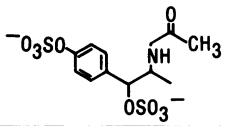
Monobactam	R	R'
SQ 26,180	CH_3—	OCH_3
SQ 26,445		OCH_3
SQ 26,812		OCH_3

FIG. 1. Structures of the monobactams SQ 26,180, SQ 26,445, and SQ 26,812.

Media and growth conditions. *C. violaceum* was grown on complex medium (1 liter of distilled water contained 4 g of NZ-amine A, 2 g of yeast extract, 1 g of NaH_2PO_4, 1 g of K_2HPO_4, 0.5 g of NH_4Cl, 0.2 g of $MgSO_4$, and 25 g of glucose; pH 6.6). For radioactive incorporation studies, a 5% inoculum of *C. violaceum* was introduced into M123GS2 medium (1 liter of distilled water contained 1 g of NZ-amine A, 0.5 g of $NaH_2PO_4 \cdot H_2O$, 0.5 g of K_2HPO_4, 0.25 g of NH_4Cl, 0.1 g of $MgSO_4 \cdot 7H_2O$, and 12.5 g of glucose; pH 6.6) or MES medium (1 liter of distilled water contained 0.15 g of K_2HPO_4, 0.05 g of NaH_2PO_4, 1 g of NH_4Cl, 0.15 g of KCl, 0.3 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $CaCl_2 \cdot H_2O$, 2.5 mg of $FeSO_4 \cdot 7H_2O$, 2 or 5 g of a carbon source, and 50 mmol of sodium morpholineethanesulfonic acid; pH 5.6). *Acetobacter* sp. was grown on complex medium (1 liter of distilled water contained 5 g of yeast extract and 10 g of glucose), and then a 1% inoculum was transferred to defined medium (1 liter of distilled water contained 2 g of glucose, 0.66 g of ammonium sulfate, 1 ml of a concentrated simple salts medium [12], and 50 mmol of phosphate buffer [NaH_2PO_4-K_2HPO_4]; pH 5.5).

A. radiobacter was grown in complex medium (1 liter of distilled water contained 5 g of yeast extract, 3 g of peptone, and 5 g of mannitol) and then in a defined medium similar to that described for *Acetobacter* sp. except that glycerol (2 g/liter) was used instead of glucose and the phosphate buffer was at pH 6; a 2% inoculum was used. Cells were routinely grown in 50 ml of medium in 250-ml Erlenmyer flasks on an orbital shaker at 300 rpm and 25°C.

Bioassay of monobactams. The culture supernatants were concentrated by lyophilization, when necessary, or were assayed directly by a disk diffusion assay with supersusceptible *Escherichia coli* SC 12,155 (1) or a strain of *Bacillus licheniformis* SC 9,262.

Separation and detection of monobactams. Monobactams were routinely resolved by high-voltage paper electrophoresis on Whatman no. 1 paper at pH 1.9 (2% formic acid, 8% acetic acid, vol/vol) with a voltage gradient of 80 V/cm for 20 min. Chromatography was performed with a solvent consisting of propanol, acetonitrile, pyridine, acetic acid, and water (40:40:30:9:36, vol/vol) (20). The detection of the monobactams was by bioassay, Rydon-Smith spray (14) (SQ 26,180 and SQ 26,812), or Cd-ninhydrin reagent (3) (SQ 26,445) in comparison with authentic standards.

Fluorography (7) of radioactive paper dipped in a 20% solution (wt/vol) of diphenyloxazole in acetone was performed with preflashed Kodak X-Omat AR film at −70°C (11). The radioactive regions were cut out and quantitated by liquid scintillation in Econofluor or, after elution, in Aquasol.

Radioactive incorporation studies. Small samples (usually 1 ml) of logarithmically growing cells (1×10^9 to 2×10^9/ml) were transferred to 12-ml culture tubes containing ^{14}C-labeled compounds (adjusted to 60 μM, 1-μCi/ml final concentration) and other test substances, when appropriate. For double-isotope incorporation studies, samples were adjusted to give 2 μCi of ^{14}C and 20 μCi of ^3H per ml. The tubes were incubated in a 25°C shaking water bath. Samples were removed at intervals ranging from 2 to 80 min, and the cells were pelleted by centrifugation (7,000 or 15,000 × *g*) for 2 min. The supernatants were removed and stored at 4 or −20°C for analysis by paper electrophoresis at pH 1.9. Radioactive monobactams and residual substrates were localized by fluorography, cut out, and then quantitated by liquid scintillation or eluted from the paper for further analysis. Preliminary studies with *C. violaceum* in M123GS2 medium indicated that >98% of most of the radioactive amino acids were taken into the cells within 5 to 10 min at the 60 μM concentration, whereas some organic acids (e.g., acetic and pyruvic) were more poorly taken up. The resulting radioactive SQ 26,180 accumulated in the supernatants for up to 20 to 30 min. Incorporation results after 20-min labeling times were compared to allow maximum uptake and incorporation and to minimize carbon atom scrambling.

To determine the origin of the methoxyl methyl group of SQ 26,445, L-[*methyl*-^{14}C]methionine (60.2 mCi/mmol) was added to the *Acetobacter* sp. culture to give a final concentration of 0.5 μCi/ml. The culture was harvested after 4 h, and the filtrate was subjected to chromatography and electrophoresis followed by water elution of [^{14}C]SQ 26,445 from the paper. This material was then heated in the presence of a molar excess of hydroiodic acid (specific gravity, 1.5). The $^{14}CH_3I$ generated in the reaction was carried in a stream of nitrogen through triethylamine, where it was trapped and counted by liquid scintillation in Aquasol.

Cell-free system. *Acetobacter* sp. was grown for 16 h in complex medium, and five 50-ml portions were harvested by centrifugation (10,000 × *g* for 10 min). The resulting pellet was suspended in 4 ml of 50 mM Tris-hydrochloride buffer, pH 8, and sonicated with cooling in ice with a Heat Systems-Ultrasonics, Inc. instrument (350 W, 20 kHz). Three 30-s periods of sonication, with 1-min intervals, were sufficient to completely break the cells. A 160-μl portion of the sonic extract (ca. 80 mg of protein per ml) was incubated with radioactive serine (0.5 mM, 1 μCi/ml of ^3H) and pyridoxal phosphate (0.1 mM) in a final volume of 200 μl for 1 h at 30°C; boiled sonic extracts

TABLE 1. Comparison of ^{14}C-precursor incorporations into [^{14}C]SQ 26,180 by *C. violaceum*[a]

^{14}C-precursor	Cellular uptake (%)	Incorporation into SQ 26,180 (%)	Relative incorporation
L-[*U*-^{14}C]serine	>98	0.28	1.00
L-[*U*-^{14}C]cysteine	90	0.21	0.75
L-[*U*-^{14}C]serine	>98	0.21	1.00
[2-^{14}C]pyruvate	ND[b]	0.17	0.80
L-[*U*-^{14}C]lactate	80	0.02	0.10
L-[*U*-^{14}C]serine	>98	0.34	1.00
L-[*U*-^{14}C]alanine	>98	0.10	0.30
L-[*U*-^{14}C]aspartate	>98	0.08	0.24
[*U*-^{14}C]glycine	>98	0.49	1.44

[a] One microcurie of amino acid (60 μM) was added to 1 ml of logarithmically growing cells. Incorporation into SQ 26,180 was measured at 20 min. Three separate experiments are shown, each with a relative incorporation value for L-[*U*-^{14}C]serine of 1.0.

[b] ND, Not determined.

were used as controls. The reaction was stopped with 50 μl of glacial acetic acid and centrifuged, and the supernatant fluid was subjected to paper electrophoresis on Whatman no. 1 paper at pH 1.9, 80 V/cm, for 20 min. Alanine and glycine products were detected by Cd-ninhydrin reagent and fluorography. Protein was determined by the biuret method (8).

RESULTS

Growth and production of monobactams. In MES medium, *C. violaceum* grew on glucose, trehalose, fructose, lactate, pyruvate, succinate, or malate as the sole carbon source. However, SQ 26,180 production (2 to 10 μg/ml) was detected only with glucose, trehalose, lactate, or pyruvate. *Acetobacter* sp. grew well on glucose, sucrose, glycerol, and ethanol and produced SQ 26,445 (ca. 50 μg/ml) on all of the carbon sources except glycerol. *A. radiobacter* grew on glucose, sucrose, and glycerol but produced detectable SQ 26,812 only on glycerol. The monobactams were produced during the log phase of growth in *Acetobacter* sp. and *C. violaceum*, whereas in *A. radiobacter*, the monobactam accumulated during the stationary phase.

In an effort to stimulate monobactam production in defined media, the effect of common L-amino acids on monobactam production was tested in all three cultures. In no instance was a significant (>twofold) stimulation seen over the concentration range of 0.2 to 10 mM with any of the common amino acids or certain combinations (2) of amino acids. Common D-amino acids were also tested with *Acetobacter* sp., with no significant stimulation of SQ 26,445 production. Cysteine (but not cystine) inhibited the growth of *C. violaceum* and *Acetobacter* sp. at 0.2 mM, and methionine inhibited SQ 26,445 production at 1 mM. The inhibition of growth by cysteine is a relatively common phenomenon which has been studied in some detail for *E. coli* (13).

Incorporation of radioactive label into SQ 26,180. Three types of labeling studies with mid-log-phase cultures of *C. violaceum* in M123GS2 medium were performed. In the first series of experiments, the uptake and incorporation of ^{14}C-labeled compounds into SQ 26,180 were compared; then, the effect of excess nonradioactive compounds on the incorporation of ^{14}C-

TABLE 2. Comparison of ^{14}C-precursor incorporations into [^{14}C]SQ 26,180 by *C. violaceum* in the presence of a fivefold molar excess of metabolites

Expt	^{14}C-precursor[a]	Additive[b]	Cellular uptake (%)	Incorporation into SQ 26,180 (%)	Relative incorporation
1	L-[*U*-^{14}C]serine	None	>98	0.28	1.00
		L-Cysteine	>98	0.67	2.39
	L-[*U*-^{14}C]cysteine	None	90	0.21	1.00
		L-Serine	90	0.14	0.67
2	L-[*U*-^{14}C]serine	None	>98	0.38	1.00
		Glycine	>98	0.40	1.05
		Pyruvate	>98	0.45	1.18
		Allylglycine	>98	0.54	1.42
		D-Cysteine	>98	1.12	2.95
3	[*U*-^{14}C]glycine	None	>98	0.37	1.00
		L-Serine	>98	0.15	0.40
	[1-^{14}C]glycine	None	>98	0.21	0.57
	[2-^{14}C]glycine	None	>98	0.51	1.38
4	[2-^{14}C]pyruvate	None	ND[c]	0.30	1.00
		L-Serine	ND[c]	0.19	0.63

[a] One microcurie of ^{14}C-labeled amino acid (50 μM).

[b] Nonradioactive amino acid (300 μM).

[c] ND, Not determined.

TABLE 3. Comparison of retentions of C-3 H atoms from serine and cystine incorporated into SQ 26,180 by *C. violaceum*

Precursor[a]	Ratio of ^3H to ^{14}C (dpm/dpm)		
	Starting mixture	Residual amino acid	SQ 26,180
L-[*U*-^{14}C]serine L-[3-^{3}H]serine	7.44	7.09	7.55
L-[3-^{14}C]serine L-[3-^{3}H]serine	7.80	7.40	6.51
L-[*U*-^{14}C]cystine L-[3,3'-^{3}H]cystine	6.66		0.94
L-[*U*-^{14}C]cystine L-[3,3'-^{3}H]cystine	6.37	6.74	1.30

[a] Amino acid concentrations were adjusted to 100 μM except for the last cystine experiment, where the concentration was 50 μM. Labeling time was 15 to 20 min.

labeled precursor candidates was tested; and third, the relative incorporation of ^3H and ^{14}C from specifically labeled mixtures of serine or cysteine was measured.

The study of the uptake and incorporation of uniformly labeled serine and cysteine showed that serine was incorporated to a greater extent than was cysteine (or cystine). Serine controls were included in subsequent experiments. Pyruvate was well incorporated in comparison with serine, whereas alanine and aspartate were not (Table 1). As pyruvate holds a central position in intermediary metabolism, it was not surprising to find that it was well incorporated into SQ 26,180; however, [^{14}C]lactate was poorly incorporated (Table 1).

To differentiate among the incorporations of serine, cysteine, and glycine into SQ 26,180 and to identify and minimize metabolite interconversions, we conducted several competition-type labeling studies. The effects of adding excess amounts of various nonradioactive compounds on the uptake and incorporation of ^{14}C-labeled precursors into SQ 26,180 were examined.

The presence of a fivefold molar excess of cysteine increased the incorporation of [^{14}C]serine more than twofold (Table 2). Conversely, excess serine depressed [^{14}C]cysteine utilization.

Pyruvate, D-cysteine, and allylglycine (a stereochemical analog of cysteine) all stimulated the incorporation of [^{14}C]serine into the monobactam to various extents (Table 2). The incorporation of radioactive glycine and pyruvate was reduced by the addition of excess serine (experiments 3 and 4, Table 2).

The results of the incorportion of [*U*-^{14}C]glycine and glycine specifically labeled at C-1 or C-2 are also shown in Table 2. [1-^{14}C]glycine was incorporated with the least efficiency. These data suggest that serine was influencing the incorporation of cysteine, glycine, and pyruvate into the monobactam nucleus, possibly by reducing their conversion to serine. The slight effect of glycine, cysteine, and pyruvate (Table 2) on serine incorporation may have been due to a more direct channeling of serine into the nucleus. Although the results in Tables 2 and 3 strongly suggest that serine is the most immediate β-lactam ring precursor, more direct evidence of serine incorporation was sought. This was provided by double-isotope labeling studies (Table 3). A mixture of [3-^{3}H]serine and either [*U*-^{14}C]serine or [3-^{14}C]serine was utilized by mid-log-phase cells of *C. violaceum*. The ratio of ^3H to ^{14}C in the SQ 26,180 product was the same as in the starting material. In contrast, a similar mixture of [3-^{3}H]cystine and [*U*-^{14}C]cystine resulted in a SQ 26,180 product with a greatly decreased ^3H-to-^{14}C ratio. Therefore, only serine was being incorporated into SQ 26,180 without oxidation or hydrogen atom exchange at C-3.

Incorporation of serine into SQ 26,445. Because SQ 26,445 (Fig. 1) has a more complex side chain than SQ 26,180, it was necessary to distinguish between the incorporation of [^{14}C]serine or [^{3}H]serine into the side chain and the incorporation of the labeled serine into the β-lactam ring of SQ 26,445. A preliminary analysis of the cell-free conversion of serine to other amino acids was, therefore, undertaken. When [3-^{14}C]serine, [*U*-^{14}C]serine, or a mixture of [3-^{14}C]serine and [3-^{3}H]serine was incubated with a crude cell-free extract of *Acetobacter* sp., together with pyridoxal phosphate, radioactive alanine was generated, presumably via pyruvate. In the cell-free double-isotope study, the radioactive alanine product had lost almost 90% of the C-3 tritium label, compared with the starting serine mixture. If this reaction sequence is functional in intact cells, the alanine portion of the side chain of SQ 26,445 could be labeled by [^{14}C]serine, but without retention of the [3-^{3}H]serine label. Thus, a decrease in the ^3H-to-^{14}C ratio in SQ 26,445 during in vivo labeling might indicate the partitioning of radioactive serine into side chain and β-lactam ring segments. Therefore, in this culture, a more detailed reaction time course was monitored.

When log-phase cells of *Acetobacter* sp. were fed a mixture of [3-^{14}C]serine and [3-^{3}H]serine, radioactive SQ 26,445 was produced. Table 4 shows the kinetics of incorporation and the ^3H-to-^{14}C ratio over the 80-min incubation period. The tritium label was efficiently retained. Therefore, it can be concluded that under these conditions serine did not label the SQ 26,445 side chain and that the C-3 hydrogens of serine were retained during incorporation into the β-lactam ring.

Incorporation of serine into SQ 26,812. When grown in a complex medium, *A. radiobacter* produces a range of monobactams (17); however, when grown in the defined medium described above, the predominant monobactam was SQ 26,812 (Fig. 1).

When [*U*-^{14}C]serine was fed to late-log-phase cells of *A. radiobacter*, there was incorporation of the ^{14}C into material which had the same electrophoretic mobility as SQ 26,812. Figure 2 shows the results of a double-isotope labeling experiment similar to those described above. Both ^{14}C and ^{3}H radioactivity in the monobactam increased during the labeling time. The ratio of ^{3}H to ^{14}C in SQ 26,812, however, remained relatively constant over an 80-min time course. This leads us to conclude that the serine was incorporated without extensive oxidation or exchange of hydrogen atoms at the C-3 serine, which we also concluded from the serine incorporation data for SQ 26,180 and SQ 26,445.

Origin of the methyl methoxyl group. L-[*methyl*-^{14}C]methionine was added to a growing culture of *Acetobacter* sp. After 4 h, a portion (8 ml) of the culture was harvested by centrifugation, and 18.4 nCi of [^{14}C]SQ 26,445 was purified by chromatography and electrophoresis. When this material was analyzed, 16 nCi (87%) of the radioactivity was recovered in the form of $^{14}CH_3I$. It appeared, therefore, that here, as with cephamycin C (20), the methyl moiety of the methoxyl group was derived from methionine.

TABLE 4. Incorporation of [3-^{3}H]serine and [3-^{14}C]serine into SQ 26,445 by *Acetobacter* sp.[a]

Time (min)	SQ 26,445 (nCi/ml)		Ratio of ^{3}H to ^{14}C
	^{3}H	^{14}C	
5	183.5	17	10.8
10	178	18	9.9
20	232.5	21.5	10.8
40	259.5	22.5	11.5
80	194.5	18	10.8

[a] Twenty microcuries of [3-^{3}H]serine and 2 μCi of [3-^{14}C]serine were added at zero time to 1 ml of growing cells to give a final concentration of 60 μM serine.

DISCUSSION

In this study, three taxonomically distinct nonfilamentous bacteria utilized various carbon sources for the production of a new class of β-lactam antibiotics, monobactams. *C. violaceum* and *Acetobacter* sp. used glucose as a carbon and energy source for monobactam synthesis but did not use glycerol. *A. radiobacter* utilized glycerol efficiently to produce monobactam, but glucose did not support monobactam synthesis. Different pathways of carbon metabolism may, therefore, lead to monobactam biosynthesis in different bacteria.

The production of the monocyclic β-lactam nocardicin A by *Nocardia uniformis* subsp. *tsuyamanensis* can be stimulated by the addition of a precursor amino acid (4, 18). However, in the studies reported here on monobactam formation, no such obvious stimulation was demonstrated in any of the producing organisms. The failure of amino acids to stimulate monobactam production under rapid growth conditions in gram-negative bacteria may be the result of the rapid interconversion and turnover of the small intracellular amino acid pools and the high degree of regulation of amino acid metabolism in such organisms (19).

A comparison of the utilization of various radioactive compounds for incorporation into monobactams also has failed to suggest a unique precursor candidate. In fact, virtually every compound tested can be incorporated into radioactive monobactams, albeit in several cases at a low level (Table 1). Serine, cysteine, and glycine, all candidates for supplying the carbon atoms of the β-lactam ring, have repeatedly shown the highest efficiencies of incorporation into SQ 26,180. However, the levels were not sufficiently different to distinguish the most immediate precursor among them.

Because the cells are actively growing during these incorporation studies, most of the added precursor amino acids must be incorporated into protein and other cell components, and only a small proportion (<1%) may be channeled into monobactam synthesis. In an attempt to overcome this problem, we made efforts to study the biosynthesis in cells when growth was reduced or stopped; however, production of monobactam was impractically low, i.e., <2 μg/ml (unpublished data).

To identify and minimize the potential rapid in vivo interconversion among these key metabolites, we performed competition-type labeling studies. The sparing effect of excess nonradioactive cysteine on [^{14}C]serine incorporation and the depressive effect of serine on [^{14}C]cysteine incorporation, without any dramatic effects on the cellular uptake, suggest a close metabolic relationship between the amino acids and identify serine as the preferred precursor (Table 2). In cell-free extracts of both *Acetobacter* sp. and *C. violaceum*, there was no evidence of serine being formed from cysteine or vice versa, although other pyridoxal phosphate-dependent products were formed (unpublished data) and such an interconversion is known (16). The stimulatory effect of allylglycine, a cysteine ana-

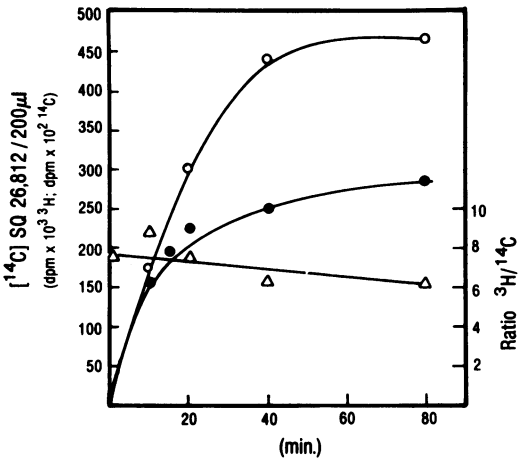


FIG. 2. Incorporation of [3-^{3}H]serine (●) and [3-^{14}C]serine (○) into SQ 26,812 by *A. radiobacter* and the ratio of ^{3}H to ^{14}C (△) over the time course.

log, on [^{14}C]serine incorporation into SQ 26,180 suggests that cysteine also may have some regulatory role in monobactam biosynthesis. However, in nonradioactive control studies with several concentrations of serine and cysteine, no increase in the final monobactam titers was seen. The effects of glycine and pyruvate on [^{14}C]serine incorporation and of serine on [^{14}C]glycine and [^{14}C]pyruvate incorporation are similar to, but not as pronounced as, the effect of cysteine. The results with glycine are consistent with an interconversion between glycine and serine (15). A more detailed study of glycine metabolism with specific ^{14}C labels has provided evidence for such activity in *C. violaceum*. The utilization of [2-^{14}C]glycine is much higher than that of [1-^{14}C]glycine in monobactam production (experiment 3, Table 2). This can be explained in terms of the more efficient entry of the C-2 of glycine into the single-carbon metabolic pool as methylenetetrahydrofolate (15). This methylenetetrahydrofolate can then condense with another glycine molecule to form serine (5). Thus, the resulting radioactive serine will receive ^{14}C-atoms by two routes in the case of [2-^{14}C]glycine, leading to the higher effective incorporation into the monobactam for [2-^{14}C]glycine than for [1-^{14}C]glycine. These differential incorporation data for glycine also make it very unlikely that an alternative mechanism involving a condensation between two molecules of glycine could give rise to the β-lactam ring.

The superior incorporation of [^{14}C]serine into SQ 26,180, together with the depressive effect of serine on the incorporation of cysteine, glycine, and pyruvate, strongly suggests that serine is the immediate precursor of the β-lactam ring and that these other metabolites enter the monobactam pathway by being converted to serine. Double-isotope incorporation studies provide evidence for intact serine incorporation without oxidation or exchange of hydrogen atoms at C-3. There is a good retention of ^{3}H from the C-3 position of serine in SQ 26,180 produced by *C. violaceum* (Table 4). In contrast, the retention of ^{3}H label from the cysteine C-3 is poor, which indicates that modification of cysteine takes place before its incorporation into SQ 26,180.

The incorporation of specifically labeled serine into the monobactam nucleus has proven to be a selective way of looking at the biosynthetic origin of the other monobactam β-lactam rings. In preliminary studies with *Acetobacter* sp., an examination of the imidazole formed when SQ 26,445 was treated with 6 N HCl showed that [^{14}C]serine was incorporated into the β-lactam ring, but it could not be concluded that the serine was first modified (unpublished data). There was an excellent retention of the tritium from [3-^{3}H]serine in the SQ 26,445 product (Table 4). A similar result was seen with the *A. radiobacter* system (Fig. 2). Thus, in the three systems studied, intact serine provided the carbon atoms of the monobactam nucleus. A similar result was described recently for the biosynthesis of the monocyclic β-lactam ring of nocardicin A, where a direct S_N2 ring closure displacement reaction was proposed (18). Experiments are in progress to elucidate the mechanism of activation of the serine hydroxy group before ring closure.

We have shown that the origin of the methyl moiety of the methoxyl group of SQ 26,445 is methionine, as in cephamycin C (20). It is not unlikely that here, as in cephamycin C, the methoxyl oxygen is derived from molecular oxygen (10) and, in view of the isolation of a desmethoxy monobactam from *A. radiobacter* (17), that the methoxyl group is added after ring closure, as in cephamycin C (9).

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